

Combining Focused Antisense Screening Technology (FAST) and Structure-based Drug Design (SBDD) for Antibacterial Discovery

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Process Flow



- Target Validation
- Rapid MOA Through Sensitized Strains
- Screening

- Increase Potency
- Expand Spectrum
- Improve Drug Properties

Overview of Rx³ Programs

All Work Grant-Supported

Program	Lead Potency MIC <i>S. aureus</i>	Assets (Target Prod. Profile)
MetRS	0.25 µg/mL	15 x-tal Structures; 4 Lead Series (linezolid)
DHFR-MetRS	0.25 µg/mL	6 x-tal Structures; 1 Lead Series (linezolid+, Cidal)
Mur A/B	2 µg/mL	4 x-tal Structures; 4 Lead Series (Broad Spectrum, Cidal)

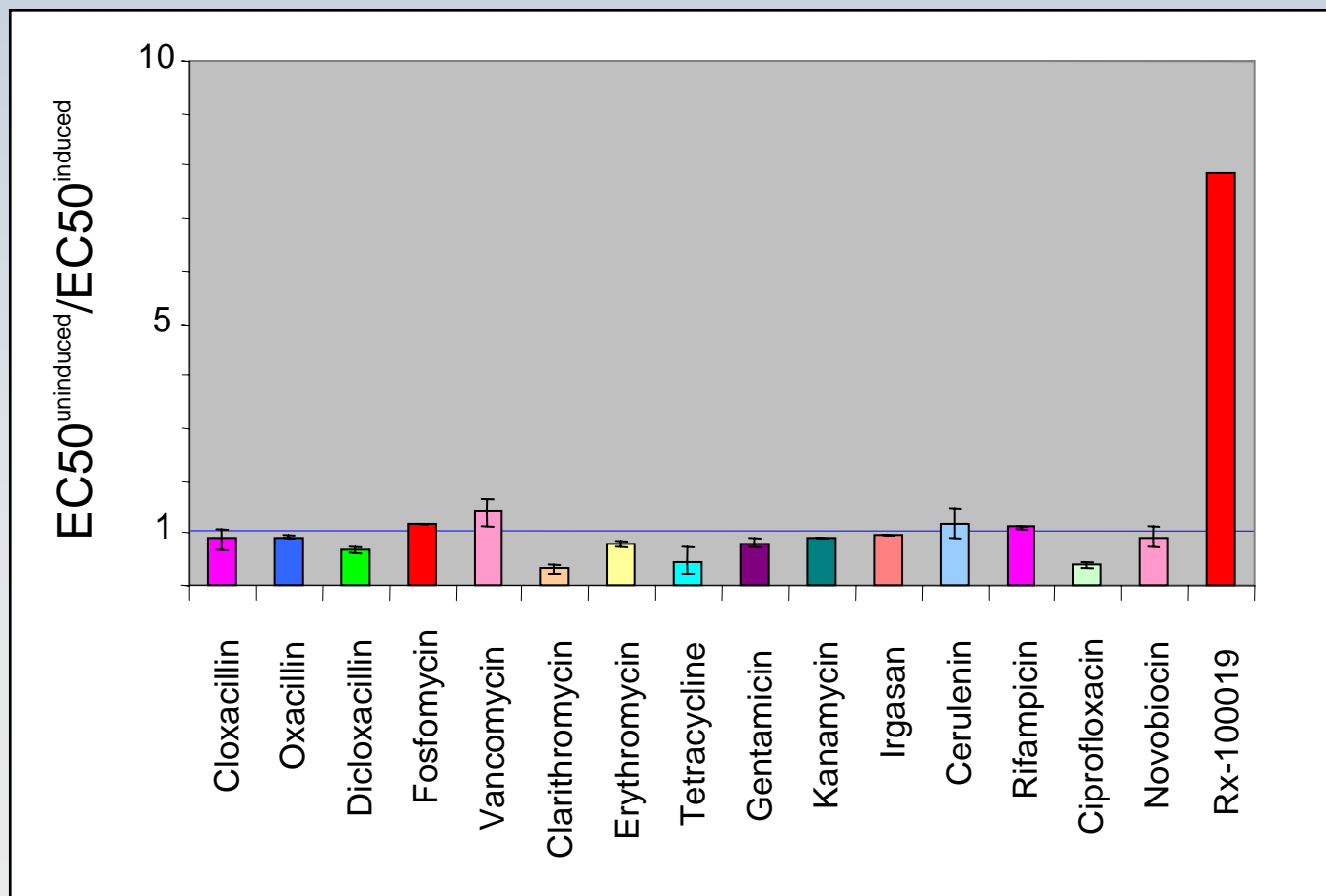


MetRS Background

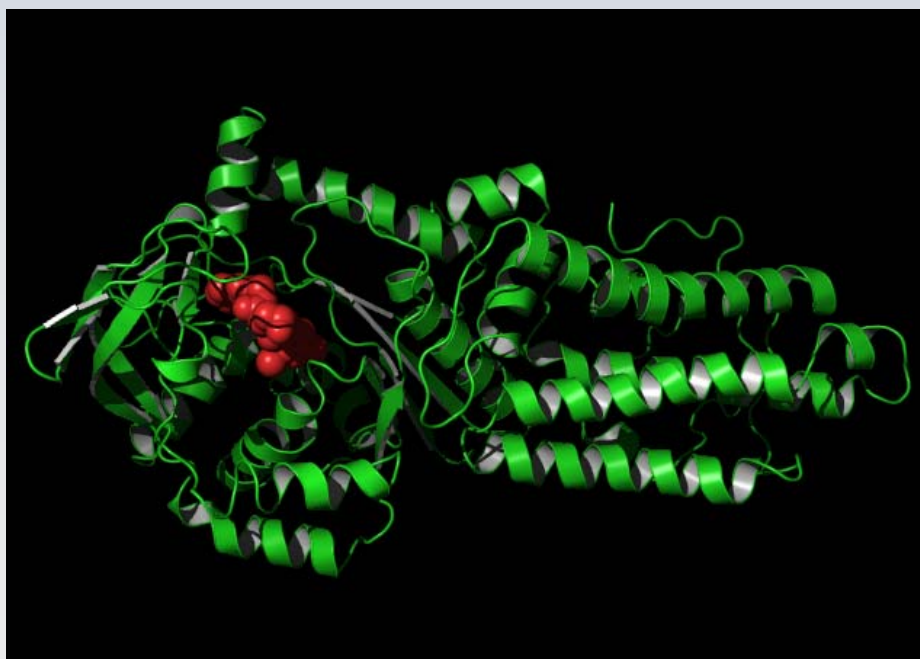
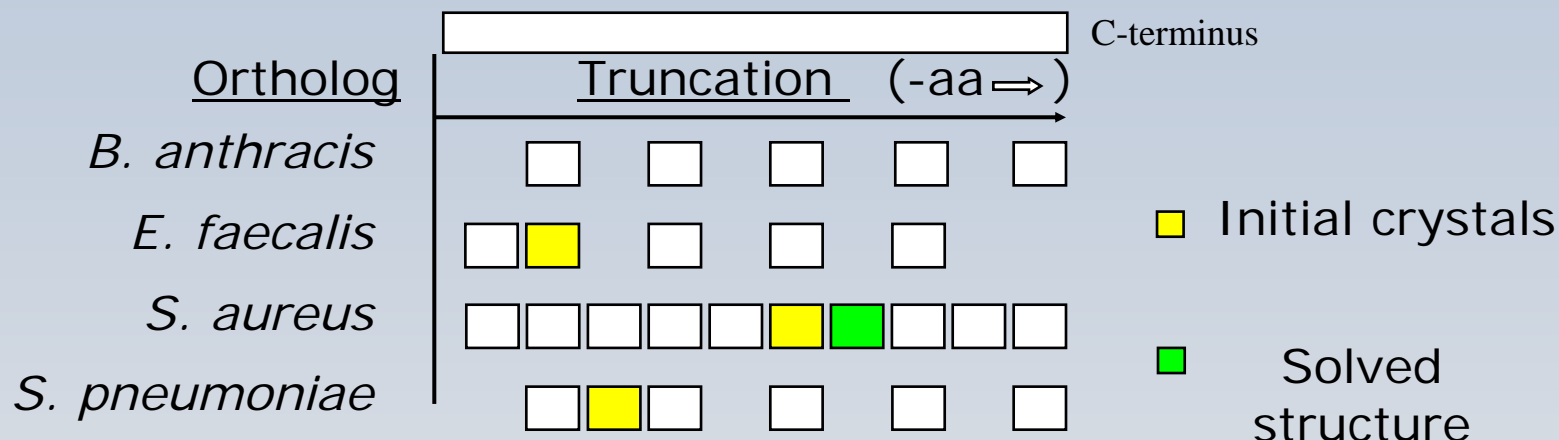
- Validated target with multiple inhibitor chemotypes.
 - Cubist, Merck, GSK, Replidyne
- Replidyne advanced a topical drug (REP-8839) into development 2006.
 - Has high serum binding.
- Gram-negative protein structure known, but Gram-positive structure not published.

metRS1 Antisense Strain Tested Against a Panel of Antibiotics

Comparing EC50 values in induced and uninduced conditions, the *metRS1* antisense strain is hypersensitive only to MetRS-targeted antibiotics



Process Used to Obtain Gram-positive MetRS Crystals

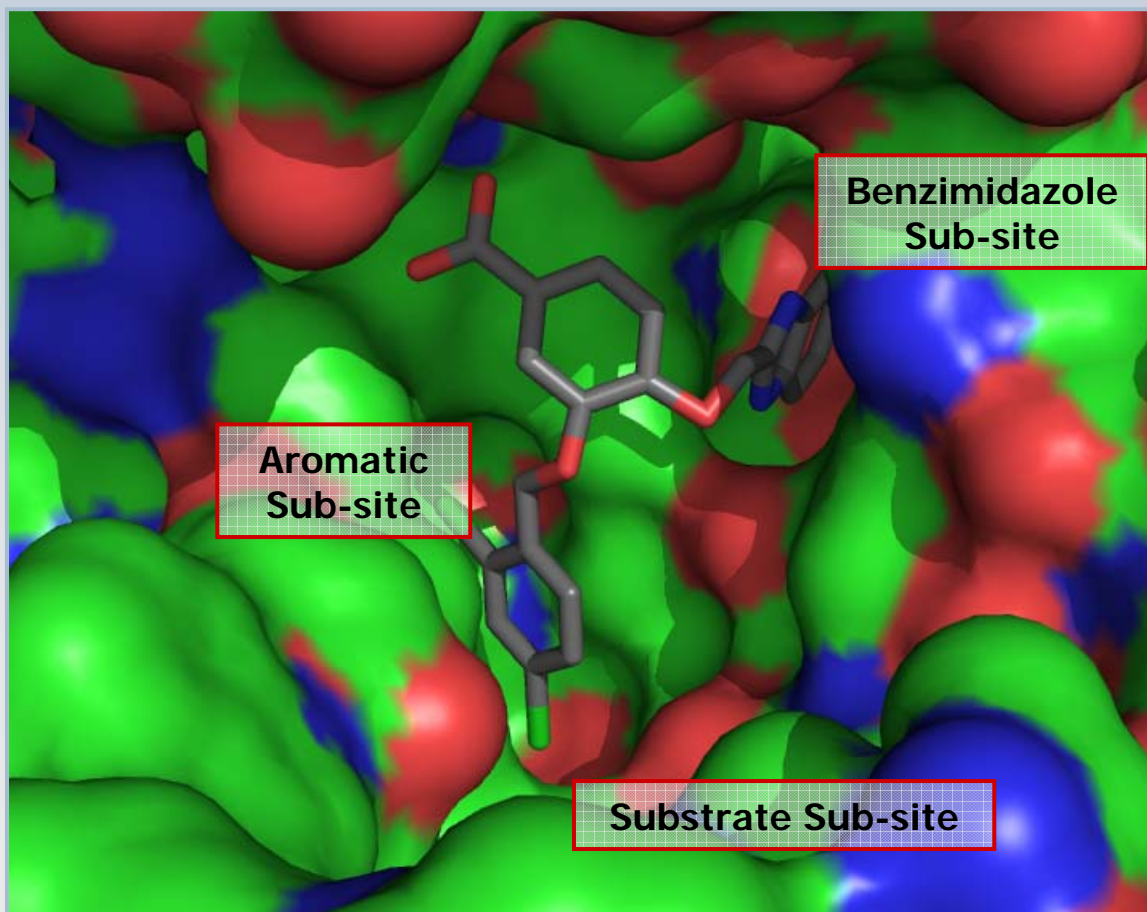


Solving the structure of Gram-positive MetRS involved thousands of crystalization experiments.

$P2_12_12_1$
 $d_{\min} = 2.2\text{\AA}$
 $R_{\text{work}} = 22.0$
 $R_{\text{free}} = 29.1$

Rx-100,019 Bound to *S. aureus* MetRS

S. aureus MetRS Offers an Unusually Rich Set of Options for Compound Optimization.



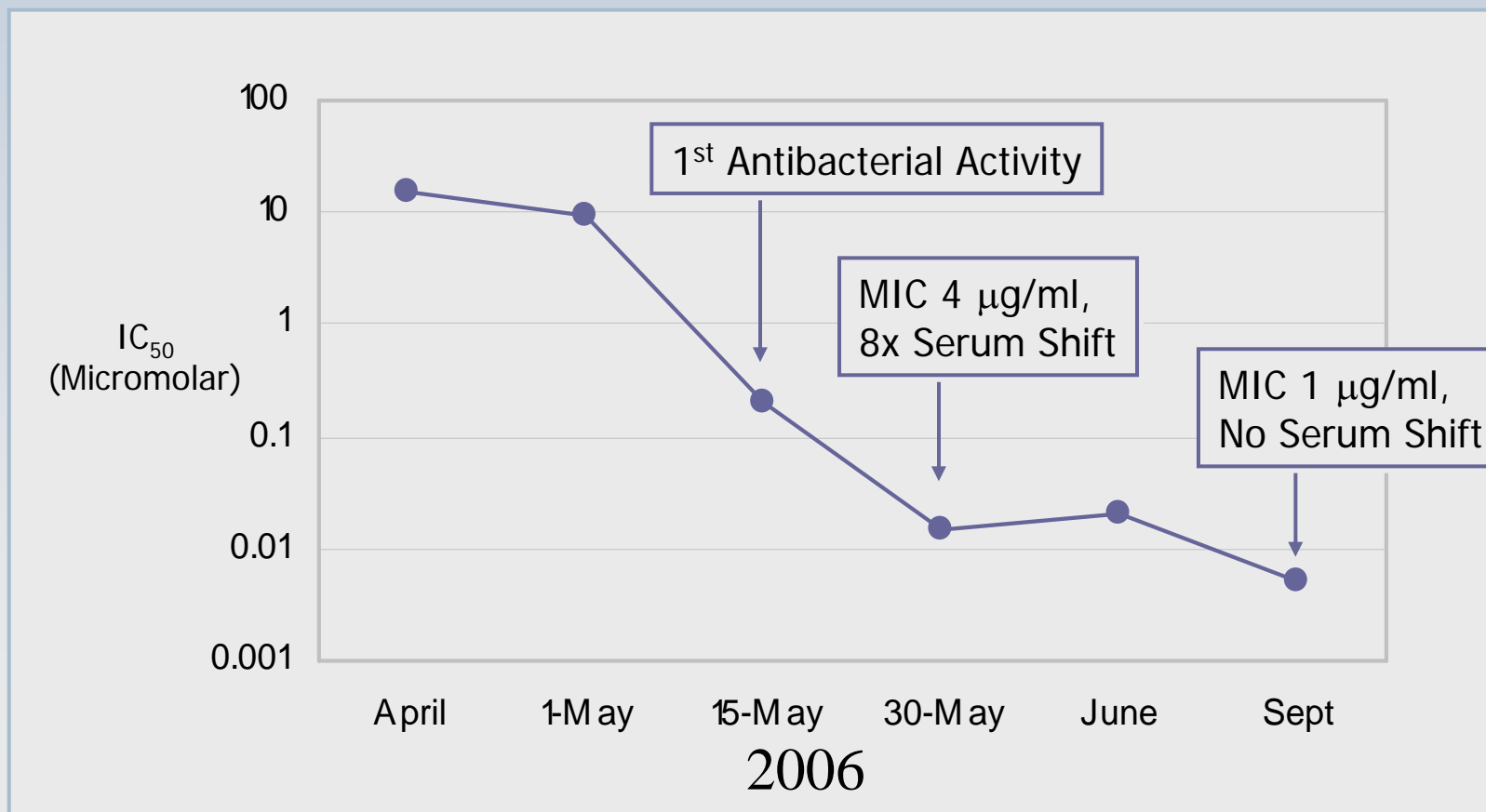
Progression of Compounds From SBDD

Virtual screening and testing followed by SBDD resulted in discovery of a new chemical series.

	Hit from virtual screen	First sub-micromolar inhibitor	First antimicrobial with MetRS MOA
IC ₅₀	20% inhibition @ 100 μ M	220 nM	32 nM
MIC	Inactive	>64 μ g/mL	4 μ g/mL

Timelines for Progress in Novel MetRS Series

Additional SBDD work has improved both potency and drug properties in a short period of time.



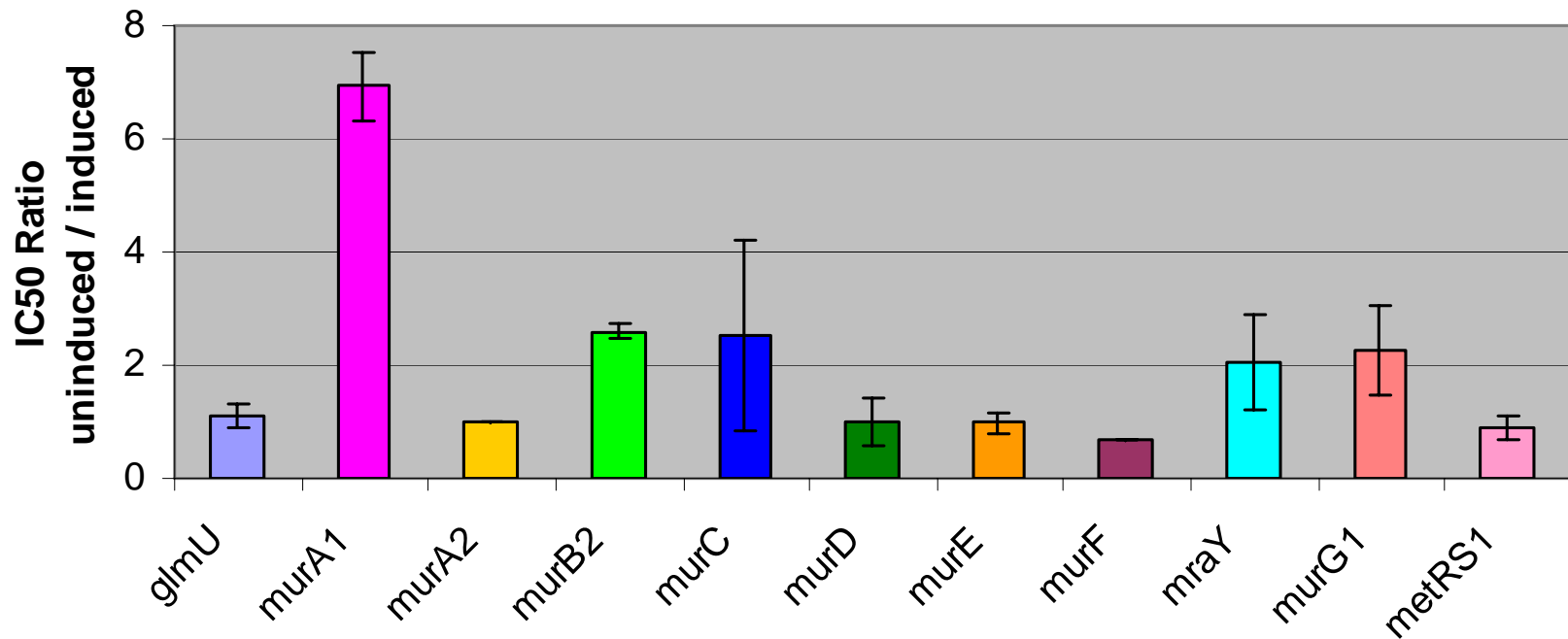
Antibacterial Spectrum of Advanced MetRS Inhibitors

Current lead spectrum and potency compare favorably to linezolid and other clinical compounds.

	MIC ($\mu\text{g/mL}$)		
STRAIN	Rx- 100472	Rx- 100473	Linezolid
<i>S. aureus</i> Smith	1	2	1
<i>S. aureus</i> +20% serum	1	2	1
MRSA	1	2	1
<i>E. faecalis</i>	0.125	0.25	2
<i>E. faecium</i> VRE	0.06	0.06	2
<i>S. pneumoniae</i>	2	1	1
<i>B. anthracis</i>	0.125	0.125	0.5

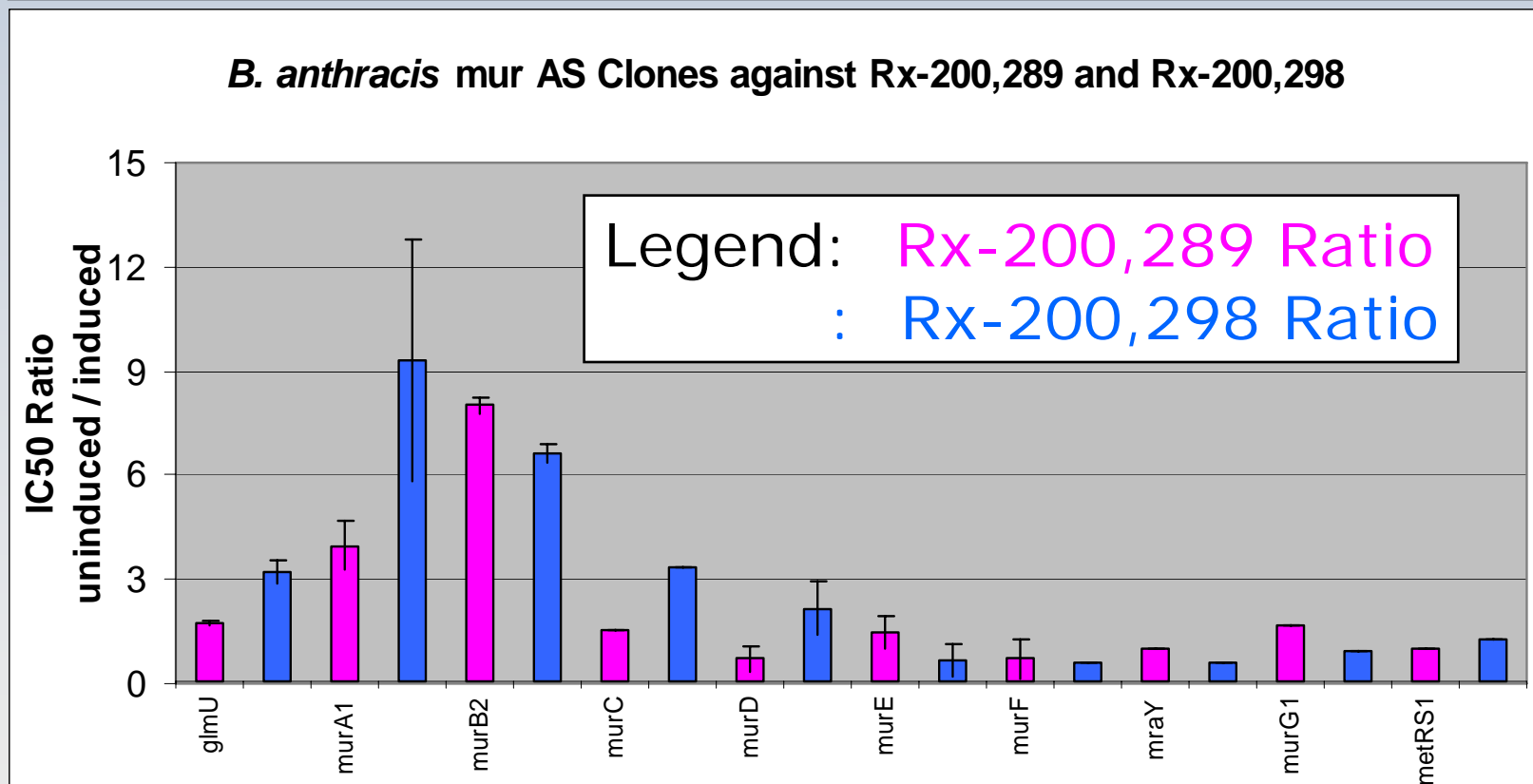
Effect of Fosfomycin on *B. anthracis* Mur Antisense Strains

The MurA-specific antibiotic fosfomycin gives a hypersensitivity signal only for the *murA1* antisense strain.



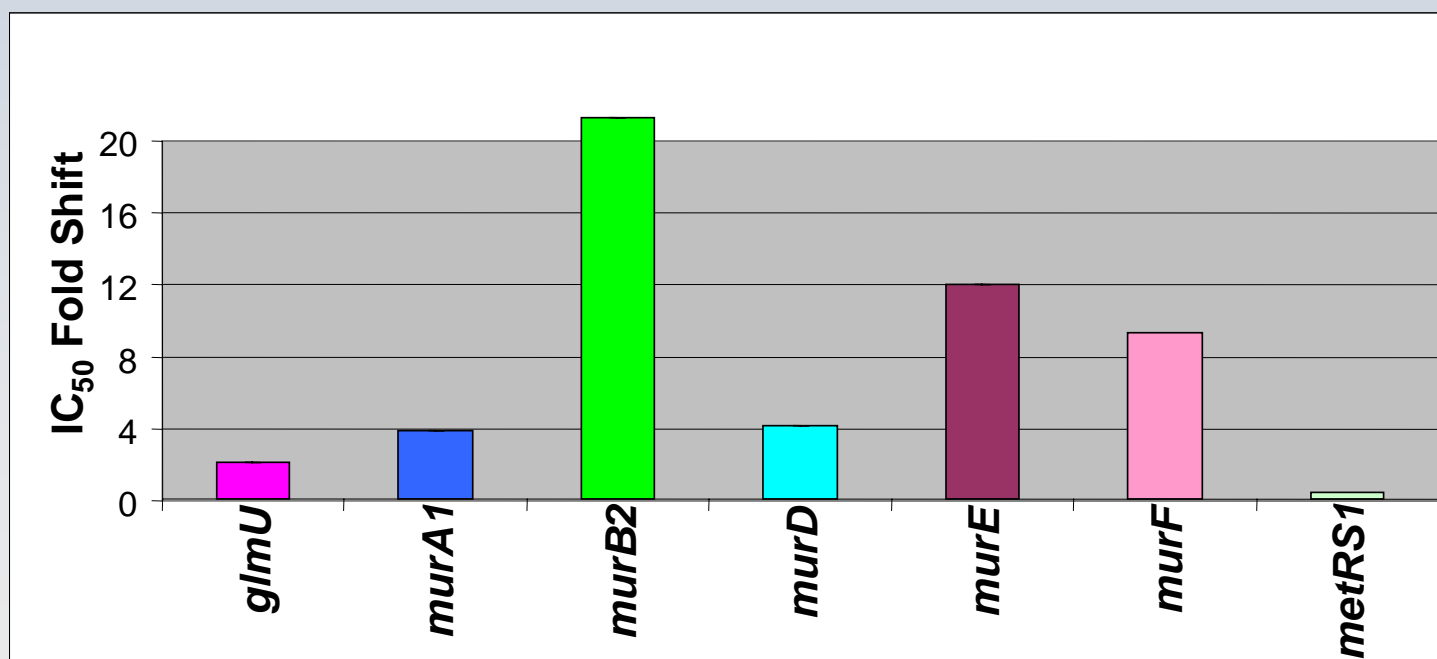
Mur antisense Panel Profile of Lead Series

Compounds in SBDD optimization show hypersensitivity signals against both *murA1* and *murB2* antisense strains.



Mur-Antisense Panel in Natural Product Screening

- 30,000 Plant Extracts
- 34 found to have antibacterial activity against Gm +
- 12 active against *B.anthraxis*
- 1 compound with cell wall specificity (MIC = 2 μ g/mL)



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The Rx³ Team

